

Inhalation Exposure Operation for Non-human Primates and Rabbits

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Topics

- Inhalation exposure components
 - Inhalation system
 - Components
 - Operational parameters
 - Plethysmography
 - Particle sizing
 - Microbiology support
 - Animal handling
- Aerosol exposure experience
 - Calculation Examples
 - Battelle LD₅₀ data summary
 - Pitfalls

Inhalation System

- Design and operational parameters modeled after USAMRIID's inhalation exposure system.
- Housed within a Class III BSC
- Inhalation exposures
 - Rabbit – muzzle only
 - Monkey – head only
- Particle size generation
 - 1-2 μm (Collison 3-jet nebulizer)
 - Liquid suspensions

Inhalation System

- Inhalation system components
 - Collison nebulizer (3-jet) – Generates agent aerosol
 - Mixing tube
 - Exposure chamber
 - Viable particle sampling port: AGI-4
 - Particle sizing sampling port: APS
 - Temperature and relative humidity probe
 - Bubbler – maintains humidity
 - Exhaust

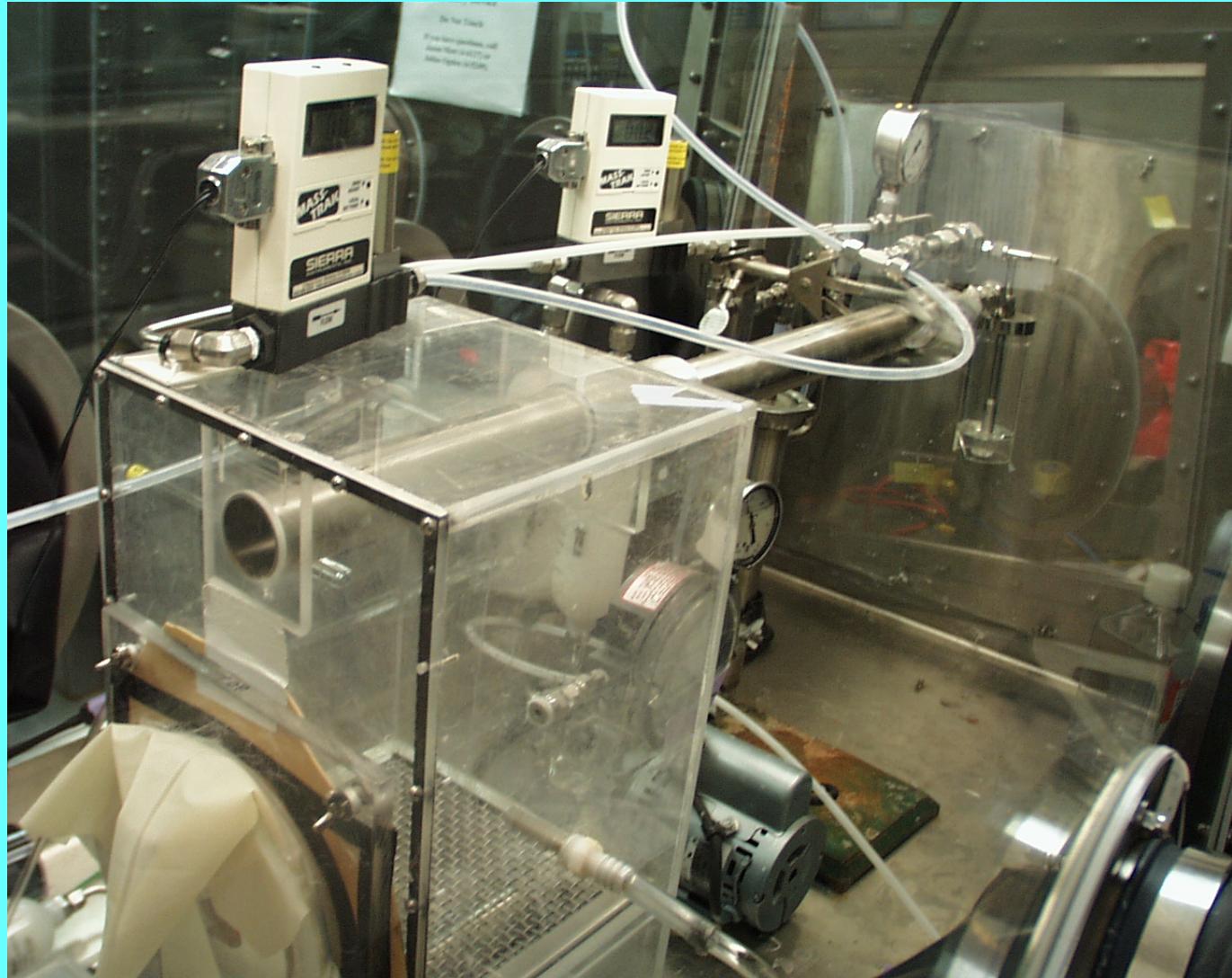
Operational Parameters

- House air pressure - ~30 psi
- Collision 3-jet nebulizer
 - 1-2 μm particle size
 - Flow rate - 7.5 lpm (Sierra mass flow controller)
 - Head-pressure - ~ 25-28 psi (Collision dependent)
- Dilution air
 - 8.5 lpm (Sierra mass flow controller)
 - 16 L total flow through system
- Exposure chamber
 - Pressure ~ 0 to -0.2 in Hg, measured using a Magnehelic
 - Volume = 0.56 cubic feet or 15.8 L
 - The neg. pressure maintains constant flow through chamber

Operational Parameters

- Viable sample collection
 - All glass Impinger (AGI-4) - sample rate of 6 lpm (critical orifice)
 - Vacuum pressure - ≥ 17 in Hg (vacuum gauge)
- Particle size collection
 - Sample rate of 1 lpm (for 30 sec)
- Exhaust
 - $\sim 10 - 11$ lpm

Photo of Inhalation Exposure System



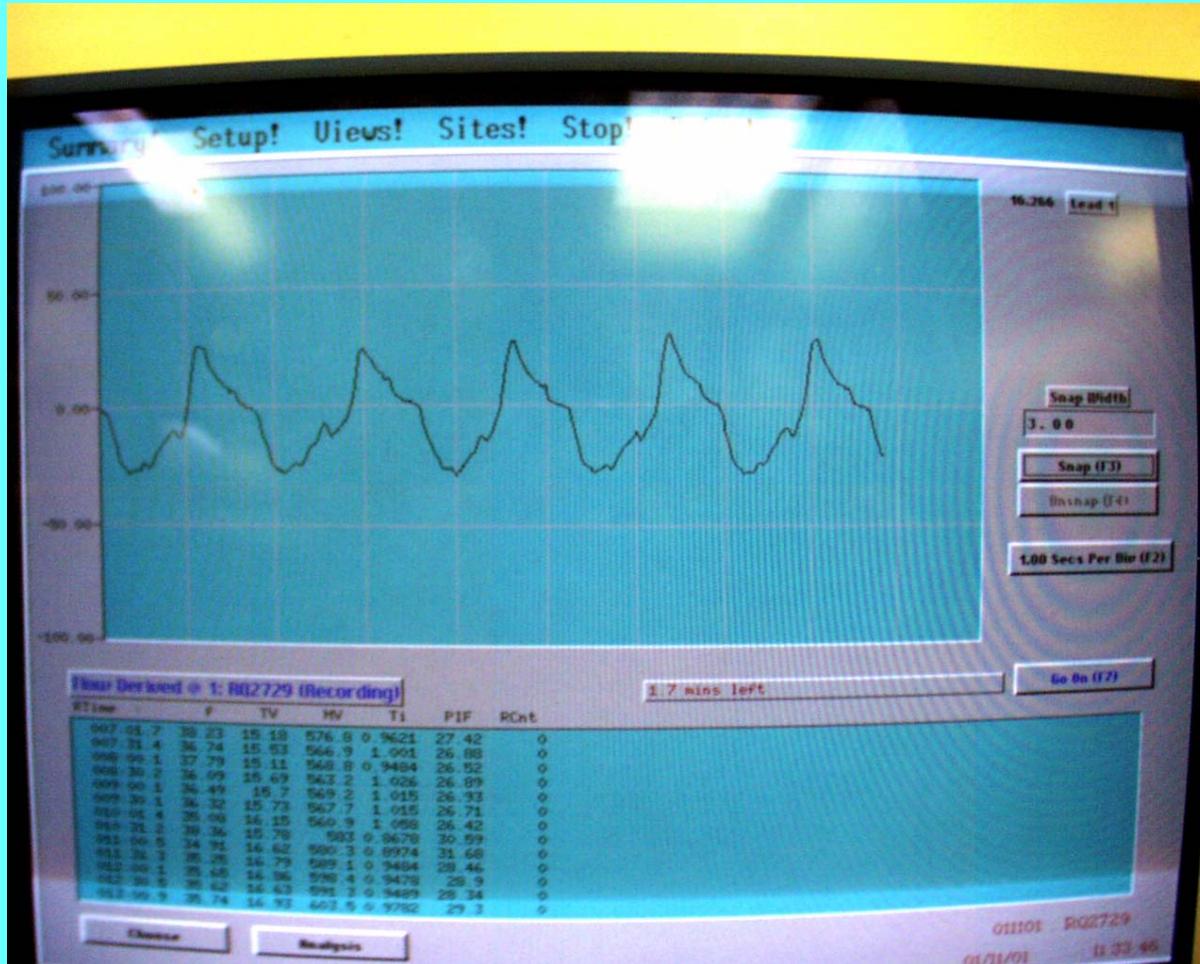
Collision 3-Jet Nebulizer and AGI-4 Impinger



Plethysmography

- Measures respiratory parameters
 - minute volume, tidal volume etc.
 - “Real-time” during exposures
- Buxco BioSystems XA – Version 1.5.7
 - Validatable
- Plethysmography boxes (sealed chambers)
- Pneumtach (Hans Rudolph)
- Transducer (Validyne, DP-45)

Buxco Data



Particle Sizing

- Real-time during exposure
- Aerodynamic Particle Sizer (APS) spectrometer 3321(TSI, Inc)
- Measures particle sizes from 0.5 to 20 μm
- Time-of-flight measurements
- Validatable

Aerodynamic Particle Sizer



System Checks

- Mass flow controllers – flow calibrated
- AGI-4 – flow calibrated with bubble meter (Buck or Gillibrator) prior to use
- Magnehelic - calibratable
- Vacuum gauge - calibratable
- Pressure gauge - calibratable
- Temp./RH probe - calibrated
- Bubble meter – calibrated
- Buxco XA – calibrated with gas-tight syringe prior to use
 - Moving to using Inspira small animal volume ventilator
- APS – particle size check with NIST Traceable PSL beads
- Aerosol system balanced prior to use – check list

Microbiology Support

- Enumerate starting material (Collison) and AGI samples
 - Spread plate technique
- Serial dilutions to get into countable range (25-250 cfu/plate)
 - Nebulizer (starting material) dilutions
 - Seven 1:10 dilutions (0.5 mL in 4.5 mL)
 - Last 3 dilutions plated in quintuplicate
 - AGI-4 sample dilutions
 - Five 1:10 dilutions (0.5 mL in 4.5 mL)
 - Last 3 dilutions plated in quintuplicate
- Up to 25-30% coefficient of variation
 - Typically ~20%
- ASTM – American Society for Testing and Materials
 - D 3870 – 91 Standard Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology
 - CV should be less than 35%

Animal Handling

- Anesthetize NHPs
- Technicians load animals into plethysmography boxes
- Post challenge observations
- Collect sera
- 1 to 2 technicians needed

Aerosol Exposure Experience

Aerosol Calculations

- $ID = (C \times V)(S \times T)^{-1}(AV)$
- Where ID = Inhaled dose (CFU)
 C = AGI concentration (CFU/ml)
 V = AGI sampler volume (ml)
 S = Sampling rate (6 L/min)
 T = Exposure time (min)
 AV = Accumulated tidal volume (L)

LD₅₀ and Spray Factor (SF) Calculation Example

Neb (cfu/mL)	AGI [] (cfu/mL)	AGI Vol. (mL)	AGI (cfu)	Sample Rate (L/min)	Sample Time (min)	Aerosol [] (cfu/L)	Total Volume inhaled (L)	Inh. Dose (cfu/animal)	Ames LD50 Equivalents	SF
9.00E+08	2.44E+06	17.00	4.15E+07	6.00	10.50	6.58E+05	3.60E+01	2.37E+07	225.74	7.32E-07
9.00E+08	2.95E+06	17.25	5.09E+07	6.00	13.10	6.47E+05	3.60E+01	2.33E+07	221.97	7.19E-07

ID: $\frac{(2.44 \times 10^6 \text{ cfu/mL})(17 \text{ mL})}{(6 \text{ L/min})(10.5 \text{ min})} = 6.58 \times 10^5 \text{ cfu/L}$

$(6.58 \times 10^5 \text{ cfu/L})(36 \text{ L}) = 2.37 \times 10^7 \text{ cfu}$

$\frac{2.37 \times 10^7 \text{ cfu}}{105,000 \text{ cfu}^*} = 225.7 \text{ LD}_{50}$

SF: $\frac{6.58 \times 10^5 \text{ cfu/L}}{(9.00 \times 10^8 \text{ cfu/mL})(1000\text{mL/L})}$

$= 7.32 \times 10^{-7}$

*1 Rabbit LD₅₀

Target Accumulated Tidal Volume Calculation

- $AC = (SF)(SC)$
- Where AC = Aerosol concentration (cfu/L)
 SF = Spray factor
 SC = Starting nebulizer conc. (cfu/L)

Total spores needed to be inhaled = $1LD_{50} \times \text{Target}$

Target Accumulated Tidal Volume Calculation

- $AC = (SF)(SC)$
- $AC = (1.2 \times 10^{-6})(1 \times 10^9 \text{ cfu/mL})(1000 \text{ mL/L})$
 $= 1.2 \times 10^6 \text{ cfu/L}$

Target Accumulated Tidal Volume Calculation

- $1 \text{ RM LD}_{50} = 55,000 \text{ cfu}$
- $(55,000 \text{ cfu})(100 \text{ LD}_{50}) = 5,500,000 \text{ cfu to inhale}$
 - $\text{AV} = \text{cfu needed to inhale} \div \text{AC}$
 - $\text{AV} = (5,500,000 \text{ cfu}) / (1.2 \times 10^6 \text{ cfu/L})$
 - $\text{AV} = 4.58 \text{ L to get } 100 \text{ LD}_{50}$

Keys are knowing with “accuracy” the SF and Starting Conc.

Target versus Mean LD50s Delivered: Published

Aerosol Days	Target Dose (LD ₅₀ s)	Mean (LD ₅₀ s)	Standard Deviation (LD ₅₀ s)	Range (LD ₅₀ s)		# Animals Challenged
				Low	High	
NG A	NG	8	3	NG	NG	58 (rm)
NG A	NG	50	28	NG	NG	32 (rm)
NG B	NG	93	63	NG	NG	42 (rm)
NG C	NG	NG	NG	255	760	15 (rm)
NG C	NG	NG	NG	161	247	3 (rm)
NG C	NG	NG	NG	239	535	10 (rm)
NG D	NG	899	62	NG	NG	22 (rm)

NG = Not Given

A - Friedlander et al. 1993. J. Inf. Dis. 167: 1239-1242

B - Ivins et al. 1998. Vaccine Vol. 18, No. 11/12, p. 1141-1148

C - Ivins et al. Salisbury Medical Bulletin – Special Supplement 87

D - Pitt et al. Salisbury Medical Bulletin – Special Supplement 87

rm = rhesus monkey

Factors Affecting Calculated Inhaled Dose LD₅₀

- Spore enumeration variability
- Spore Lot/Spores
 - Aggregations/clumping
 - “Stickiness”
 - Debris
 - Adherence to glass and plastics
 - Spores are not your usual laboratory microbe
- Exposure time
- AGI sample enumeration

Spore Enumeration Variability Example

Nebulizer starting concentration and AGI sample enumeration can vary
By up to 25-30%

AGI [] (cfu/mL)	Inh. Dose (cfu/animal)	Ames LD50 Equivalents
1.55E+06	1.58E+07	150
2.06E+06	2.10E+07	200
2.58E+06	2.63E+07	250
3.09E+06	3.15E+07	300
4.12E+06	4.20E+07	400
5.15E+06	5.25E+07	500
1.44E+06	1.47E+07	140
2.06E+06	2.10E+07	200
2.68E+06	2.73E+07	260
2.88E+06	2.94E+07	280
4.12E+06	4.20E+07	400
5.36E+06	5.46E+07	520

Spore Lot Variability Example

<u>Lot #</u>	<u>SF</u>	<u>TV to Inhale (L)</u>	<u>Target 100LD₅₀</u>	
B5	3.30×10^{-6}	1.87	100	100
B8	9.90×10^{-7}	6.24	334	30
B10	1.42×10^{-6}	4.35	233	43
B11	1.20×10^{-6}	5.15	275	36

B4 n=3

B8 n=15

B10 n=5

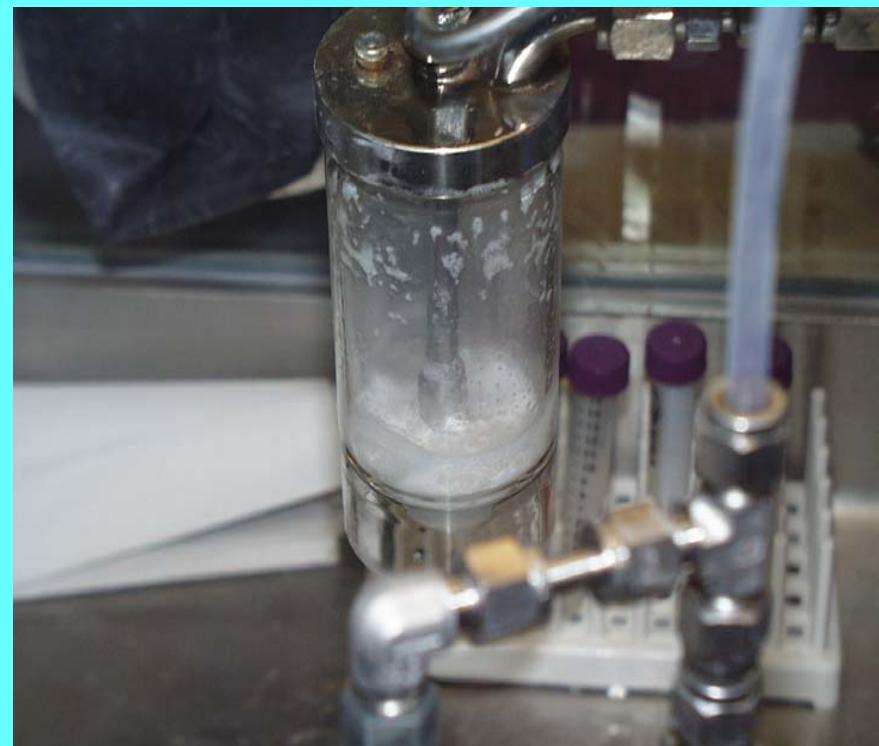
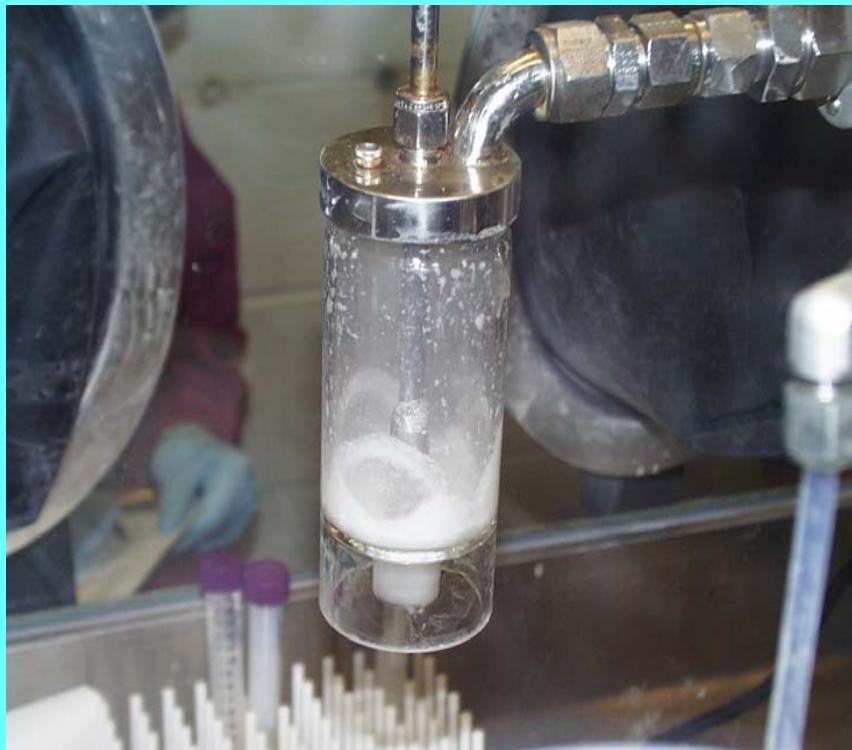
B11 n=3

Inhalation Exposure Pitfalls

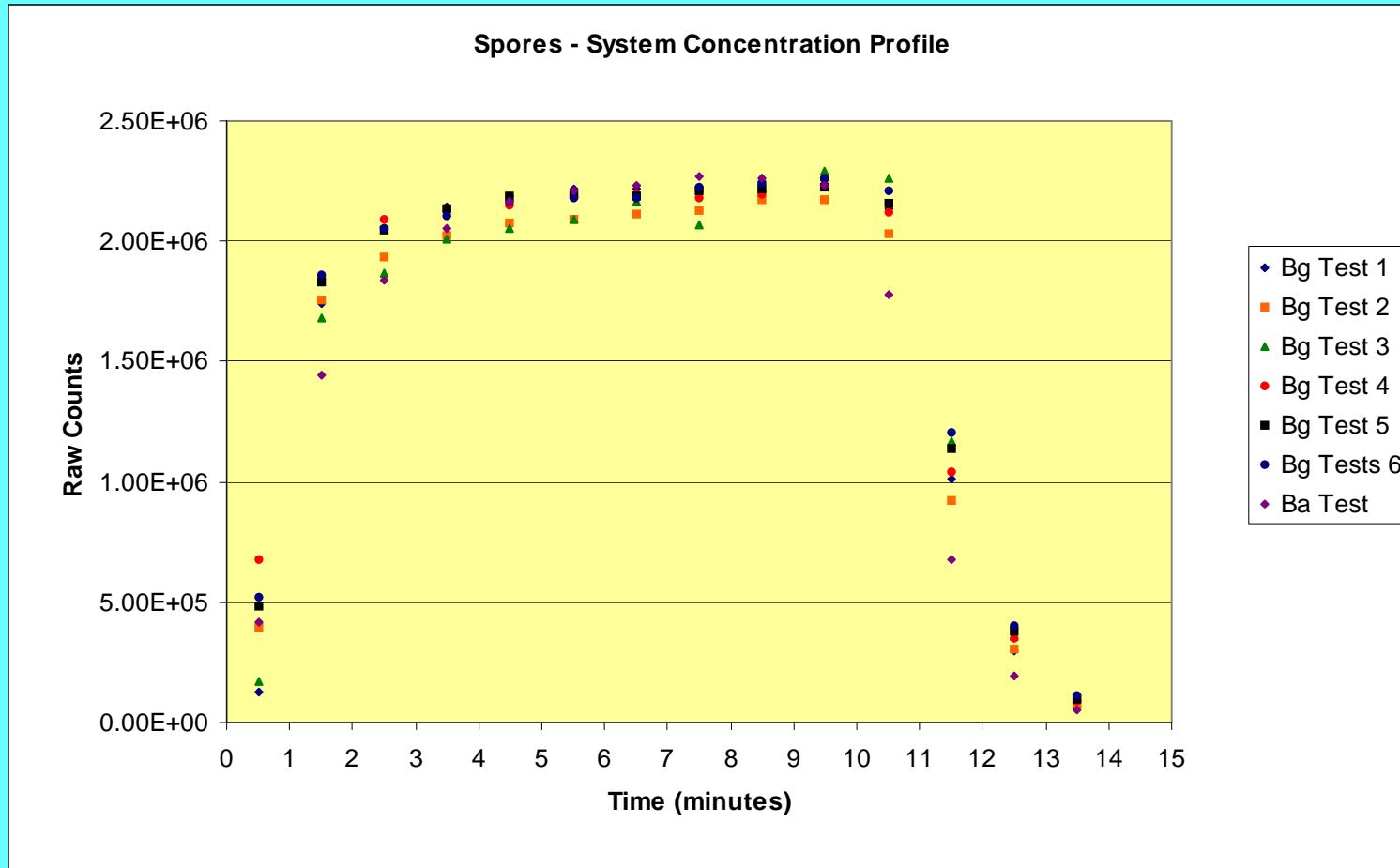
■ Spore Foaming

- Collision head-pressure – Too high
- Spore lot phenomenon
- Collision tip is too deep in well (~2-3 mm below fluid level)

3-Jet Collision Nebulizer – Spore Foaming



System Atmosphere Concentration Profile



- Effect of exposure time

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